VII. CLAIMING

What is claimed is:

1. The procedure for cloning human SMN gene based on the reverse transcription (RT) and the polymerase chain reaction (PCR) using the synthesized oligonucleotides (SEQ ID NO. 1) for RT, and (SEQ ID NO. 2) and (SEQ ID NO. 3) respectively for PCR, comprising:

- Isolating SMN-mRNA;
- Performing RT reaction using the synthesized oligonucleotide 5' TGGCAGACTTAC 3' (SEQ ID NO. 1) under the following conditions: 90°C for 2 minutes; 0°C for 1 minute; 25°C for 10 minutes; 42°C for 45 minutes;
- Performing PCR reaction using the synthesized oligonucleotides 5' ATGGCGATGAGCAGCGG 3' (SEQ ID NO. 2) and 5' TTAATTTAAGGAATGTGAGCAC 3' (SEQ ID NO. 3) under the following conditions: Denaturating at 94°C for 1 minute; annealing at 55°C for 2 minutes; elongating at 72°C for 1 minute each cycle, for 35 cycles.
- Ligating the PCR product of SMN gene into the PCR II plasmid vector (SEQ ID NO. 4) and introducing the ligation product in INVα F' E. Coli competent cells;
- Screening for inserts based on the presence of white colonies that results in the selection of the vector (**1**) (SEQ ID NO. 4 / SMN-cDNA).

2. The procedure for the construction of expression plasmids using the pFastBac HTb baculovirus transfer vector of the Bac-to-Bac baculovirus expression system and the pBlueBacHis2 A baculovirus transfer vector of the Bac-N-Bac baculovirus expression system for the purpose of obtaining SMN recombinant protein in insect cells, comprising:

2.1. Using the pFastBac HTb baculovirus transfer vector of the Bac-to-Bac baculovirus expression system:

- Digesting the pFastBac HTb baculovirus transfer vector (SEQ ID NO. 5) with BamHI and XhoI followed by dephosphorylation with calf intestinal alkaline phosphatase;

- Digesting the vector (**1**) (SEQ ID NO. 4 / SMN-cDNA) with BamHI and XhoI and isolating the resulting fragment containing the cDNA encoding sequences of SMN protein, SMN- cDNA;

- Ligating the SMN-cDNA fragment to the pFastBac HTb vector (SEQ ID NO. 5) and introducing the ligation product in INVα F' E. Coli competent cells;

- Screening for inserts based on the presence of white colonies, as a result of which the vector (**2**) (SEQ ID NO. 5 / SMN-cDNA) is selected;

- Introducing the vector (**2**) in DH10Bac E. Coli competent cells of the Bac-to-Bac baculovirus expression system kit;

- Screening for recombinant bacmids in DH10Bac E. Coli based on the presence of white colonies, then verifying the presence of SMN-cDNA's insert in the recombinant bacmids by PCR amplification using the M13 forward (-40) and M13 reverse primers, as a result of which the recombinant bacmid (**3**) is selected;

2.2. Using the pBlueBacHis2 A baculovirus transfer vector of the Bac-N-Bac baculovirus expression system:

- Digesting the pBlueBacHis2 A baculovirus transfer vector (SEQ ID NO. 6) with BamHI and XhoI followed by dephosphorylation with calf intestinal alkaline phosphatase;

- Digesting the vector (**2**) (SEQ ID NO. 5 / SMN-cDNA) with BamHI and XhoI and isolating the resulting fragment containing the cDNA encoding sequences of SMN protein, SMN-cDNA;

- Ligating the SMN-cDNA fragment to the pBlueBacHis2 A vector (SEQ ID NO. 6) and introducing the ligation product in INVα F' E. Coli competent cells;

- Screening for inserts based on the presence of white colonies, as a result of which the vector (**4**) (SEQ ID NO. 6 / SMN-cDNA) is selected.

3. The procedure for the construction of expression plasmids using the pET-28a (+) bacterial transfer vector of the prokaryotic expression system for the purpose of obtaining SMN recombinant protein in bacteria, comprising:

- Digesting the pET-28a (+) bacterial transfer vector (SEQ ID NO. 7) with BamHI and XhoI followed by dephosphorylation with calf intestinal alkaline phosphatase;

- Digesting the vector (**2**) (SEQ ID NO. 7 / SMN-cDNA) with BamHI and XhoI and isolating the resulting fragment containing the cDNA coding sequences of SMN protein, SMN-cDNA;

- Ligating the SMN-cDNA fragment to the pET-28a (+) bacterial transfer vector (SEQ ID NO. 7) and introducing the ligation product in INVα F' E. Coli competent cells;

- Screening for inserts based on the presence of white colonies, as a result of which the vector (**5**) (SEQ ID NO. 7 / SMN-cDNA) is selected.